Also known as

M-H Agar

Specification

Recommended medium used to perform Antibiotic and Sulphonamide sensitivity testing with pathogenic microorganisms from clinical specimens, according to the Kirby-Bauer and Ericsson methodology.

Formula * in g/L

Peptone.. 17,5
Beef infusion solids.. 2,0
Starch.. 1,5
Agar.. 17,0

Final pH 7.3 ±0,1 at 25 ºC

* Adjusted and /or supplemented as required to meet performance criteria

Directions

Add 38 g of powder to 1 L of distilled water and let it soak. Bring to the boil to dissolve the medium completely. Sterilize in the autoclave at 121ºC for 15 minutes. Note: If the medium is to be used with added blood, it is advisable to add 5 g / L of sodium chloride to prevent spontaneous haemolysis.

Description

Mueller-Hinton Agar was originally designed for the primary isolation of meningococci and gonococci.
With the addition of blood it becomes an optimal medium for the growth of Neisseria. It is also more effective if reheated and turned into Chocolate Agar. It should not be re-melted or reheated once blood has been added to it. For the culture of *Neisseria* the best results are obtained if incubation is carried out in a moist chamber with a CO_2 enriched atmosphere (5 to 8% CO_2).

Technique

Mueller-Hinton Agar has proved to be one of the most efficient media for use in anti-bacterial susceptibility testing. Without the addition of blood it can be used for sulfonamide sensitivity testing since it is free from most of its antagonists (nucleotides, etc.). If this type of assay is conducted, the zones of inhibition should be examined after 12-18 hours, before overgrowth occurs, since after 24 hours it can interfere with the sulphonamide sensitivity test. Using a small inoculum will help the early formation of zones of inhibition. The inoculum should be 100 to 300-fold smaller than that used in the testing of other antibiotics.
In 1970 the WHO proposed this medium for antibacterial sensitivity testing, and it has been widely used since then. Sensitivity testing can be conducted by a variety of techniques, both on solid and liquid media. The most commonly used method in routine work is that derived from Kirby-Bauer and recommended by the American Association of Clinical Pathologists.
The Kirby-Bauer method is a precise, semi-quantitative testing system. It uses Mueller-Hinton Agar and disks with a high antibiotic concentration. The inoculum is first standardized using a MacFarland standard, then the plate is inoculated with a swab dipped in the standardized suspension, and final ly the disks are arranged properly equidistant from each other on the plate and then incubated (see the table).
Some authors suggest that the inoculum should be modified by introducing a double layer of inoculated medium. This system undoubtedly provides sharper and more defined zones of clearing of inhibition. Plates are incubated à 37ºC overnight and then the zones of inhibition are measured. Results are reported in terms of Resistant, Moderately Resistant (Intermediate) and Sensitive strains (See table).
The Ericsson technique, which has been adopted in most European countries, uses a standardized culture medium (Mueller-Hinton), a standardized quantity per plate (25 mL on 9 cm diameter plates) and standardized inoculum concentration.
The fresh culture suspension used is incubated for 18 hours is in liquid medium and is then diluted accordingly. So as to ensure the appropriate amount of growth on the agar.
Suggested Dilutions:
- Enterobacteria - Pseudomonas: dilution of 1/300.
- Staphylococcus - Enterococcus: dilution of 1/300.
- Streptococcus - Haemophilus: dilution of 1/10.

The plate is seeded by flooding its surface. The excess inoculum is removed with a sterile pipette and the antibiotic disks are arranged properly on the plate. A pre-diffusion period of 30-60 minutes is allowed prior to incubation so that the antibiotic can slowly diffuse before growth. After incubation à 37ºC for 12-18 hours, the zones of inhibition are measured and the Assay Regression Curves referenced. Results are reported in terms of Sensitive or Resistant or as Minimum Inhibitory Concentration (MIC) values.
The Ericsson technique undoubtedly offers more precision and reliability than the Kirby-Bauer. Nevertheless, the Kirby method, which is semi-quantitative, is much simpler and easier to perform in everyday practice. The Ericsson technique is highly recommended where high efficacy and precision are required.
Mueller-Hinton Agar fulfils the requirements for microbial sensitivity tests and the basic characteristics are verified in every batch. Nevertheless some variation in results can sometimes occur. Please note the following factors that can be a source of variability:
1. Since the nutritional requirements of organisms vary, some strains may be encountered that fail to grow or grow poorly on the medium.
2. Factors such as: inoculum size, rate of growth, medium formulation and pH, length of incubation and incubation envferment, disk content and drug diffusion rate, and measurement of endpoints can all affect the results.
Therefore, strict adherence to protocol is required to ensure reliable results.
3. Disk diffusion susceptibility testing is limited to rapidly growing organisms. Drug inactivation may result from the prolonged incubation times required by slow growing organisms.
4. Media containing excessive amounts of thymidine or thymine can reverse the inhibitory effects of sulphonamides and trimethoprim, causing zones of growth inhibition to be smaller or less distinct.
5. Variation in the concentration of divalent cations, primarily calcium and magnesium, affects results of amino-glycoside, tetracycline, and colistin tests with Pseudomonas aeruginosa isolates. A cation content that is too high reduces zone sizes, whereas a cation content that is too low has the opposite effect.
6. When Mueller-Hinton Medium is supplemented with blood, the zone of inhibition for oxacillin and methicillin may be 2 to 3 mm smaller than those obtained with unsupplemented agar. Conversely, sheep's blood may markedly increase the zone diameters of some cephalosporins when they are tested against enterococci.
Sheep's blood may cause indistinct zones or a film of growth within the zones of inhibition around sulphonamide and trimethoprim disks.
7. Mueller-Hinton Medium deeper than 4 mm may cause false-resistant results, and agar less than 4 mm deep may be associated with a false-susceptibility results.
8. A pH value outside the range of 7,3 ± 0,1 may adversely affect susceptibility test results. If the pH is too low, amino-glycosides and macrolides will appear to lose potency; others may appear to have excessive activity.
The opposite effects are possible if the pH is too high.
9. When Mueller-Hinton Medium is inoculated, no droplets of Humidité should be visible on the surface or on the Petri dish cover.
10. Mueller-Hinton Medium should be inoculated within 15 minutes after the inoculum suspension has been adjusted.
11. The zone of inhibition diameters of some drugs, such as the macrolides, amino-glycosides and tetracyclines, are significantly altered by CO_2. Plates should not be incubated in an increased CO_2 atmosphere.

For further information on the performance of the antibiotic disk susceptibility test refer to the M2-A9 CLSI (formerly NCCLS) Monograph, EUCAST and others.

Quality control

Incubation temperature: 35 °C ±1.0 **Incubation time:** 18 ± 2h

Inoculum: Inoculate the entire agar surface and add antibiotic disks according to EUCAST guidelines

Microorganism	Growth	Remarks
Staphylococcus aureus ATCC® 29213	Good	-
Escherichia coli ATCC® 25922	Good	-
Pseudomonas aeruginosa ATCC® 27853	Good	-
Enterococcus faecalis ATCC® 29212	Good	-
Streptococcus pneumoniae ATCC® 49619	Good	With 5% Blood + NAD (20 mg)

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Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4 ºC to 30 ºC).